Remarks

Reconsideration of this Application is respectfully requested.

Upon entry of the foregoing amendment, claims 1-3, 5-12 and 19-21 are pending in the application, with claim 1 being the sole independent claim. Claims 4, 13-18 and 22-26 have been cancelled without prejudice to or disclaimer of the subject matter therein. Claims 1-3 and 5-7 have been amended. These changes are believed to introduce no new matter, and their entry is respectfully requested.

Based on the above amendments and the following remarks, Applicant respectfully requests that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

Election

In response to the Restriction Requirement listing Inventions I-IV, Applicant hereby confirms the election to prosecute claims directed to Invention I, claims 1-12 and 19-21. This election is made with traverse.

Rejections under 35 U.S.C. § 112

The Examiner has rejected claims 1-3, 5 and 6 under the second paragraph of 35 U.S.C. § 112 as being indefinite on the basis that the term "about" renders the scope of the claims unclear. Applicant respectfully traverses this rejection

Solely in an effort to advance the prosecution of the application, Applicant has amended claims 1-3, 5 and 6 to delete the term "about." Withdrawal of the rejection is respectfully requested.

Rejections under 35 U.S.C. § 103

The Examiner has rejected claims 1-12 and 19-21 under 35 U.S.C. § 103(a) as being unpatentable over Asmus *et al.* (Asmus) in view of Watson *et al.* (Watson) and further in view of the Practical Engineering reference. Applicant respectfully traverses this rejection.

Asmus teaches that solutions containing $\geq$ 0.25 mL methacholine diluted with normal saline are stable up to 2 weeks when stored at 2 to 8° C, and that the pH of these solutions is slightly acidic. Asmus also teaches that solutions of methacholine buffered at a pH of approximately 8.3 are stable up to 9 months when stored frozen. Asmus also teaches that the relatively high pH (approximately 8.3) has been reported to enhance degradation of methacholine at room temperature.

Watson discloses that methacholine chloride solutions undergo hydrolysis if the pH exceeds 6. In support of this statement, Watson tested methacholine solutions having a pH of 4, 5 and 6. There is no distinction made in Watson as to any improved stability observed in solutions having a pH in the range of 4 to 5. See Table 1, page 590, and the first full paragraph on page 591.

Applicant asserts that the methacholine chloride solutions disclosed in Watson cannot be considered to be "inhalable." As set out in the present application, the levels of

the acetate and citrate buffers must be sufficiently low to not independently cause bronchoconstriction or bronchoirritation. Amended claim 1 recites that the buffer solution is an acetate buffer solution containing less than 0.2% acetate or a citrate buffer solution containing less than 0.1% citrate. These levels do not independently cause bronchoconstriction or bronchoirritation and are within acceptable levels of pharmaceutically acceptable buffering agents as provided by government regulatory agencies. For example, the United States' Food and Drug Administration has an Inactive Ingredient Guide (IIG) (accessible to the public at http://www.accessdata.fda.gov/scripts/cder/iig/index.cfm) that provides a listing of excipients with maximum acceptable concentration amounts (see, for example, Example 2 of the present application).

The pH 4 methacholine chloride solution disclosed in Watson contains 0.02 M acetic acid plus sodium acetate, which is equivalent to greater than 0.3% acetate.[1] Thus,

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1 The percent acetate was calculated as follows. At page 589 of Watson et al., the pH 4 buffer is identified as "acetic acid and 0.02 M sodium acetate". The concentration of acetic acid used = x M, and is calculated as follows:

$pH = pK_a + \log [salt]/[acid]$ (Henderson-Hasselbach Equation)

$4 = 4.75 + \log [0.02M]/[x]$ (pK_a for acetic acid = 4.75)

$-0.75 = \log [0.02M]/[x]$

Therefore x = 0.035 M

The total concentration of acetate in the pH 4 buffer disclosed ≈ 0.055 M

The molecular weight of acetate = 59 g/mol

Therefore, the percent acetate = 0.055 M × 59 g/mol ÷ 10

= 0.32 g/100 ml = 0.32 %

the level of acetate included in the acetate-based solution disclosed by Watson is significantly higher than the amount of acetate in the presently claimed formulation. As demonstrated in Example 7 of the present application, a methacholine chloride solution containing less than 0.2% acetate buffer caused bronchoconstriction in a subject to the same degree as caused by methacholine chloride (at the same concentration) in saline alone. Thus, the acetate buffer of in the present invention does not independently cause bronchoconstriction or bronchoirritation.

The pH 5 methacholine chloride solution disclosed in Watson contains 0.03 M citric acid plus sodium citrate, which is equivalent to greater than 1.04% citrate.[2] As indicated in the IIG, inhalable products should not contain more than 0.6% citrate. Clearly the amount of citrate used in the Watson formulation is significantly greater than the amount acceptable for an inhalation product. In contrast, the amount of citrate

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2 The percentage of citrate was calculated as follows. At page 589 of Watson et al., the pH 5 buffer is identified as "citric acid and 0.03 M sodium citrate". Thus, the concentration of citric acid used is x M, and is calculated as follows:

$pH = pK_a + \log [salt]/[acid]$ (Henderson-Hasselbach Equation)

$5 = 4.76 + \log [0.03M]/[x]$ (Using pK_{a2} for citric acid = 4.76)

$0.24 = \log [0.03M]/[x]$

Therefore x = 0.025 M

The total concentration of citrate in the pH 5 buffer disclosed $\approx$ 0.055 M

The molecular weight of citrate = 189 g/mol

Therefore, the percent citrate = 0.055 M × 189 g/mol ÷ 10

= 1.04 g/100 ml = 1.04 %

included in the presently claimed formulation is in an amount that is acceptable for inhalation.

There is nothing in Asmus, taken alone or in combination with Watson, that suggests that the use of acetate or citrate buffers having concentrations of acetate or citrate suitable for inhalation would inevitably result in stable formulations of methacholine chloride. Buffering capacity is the ability of a buffer to resist changes in pH. There are two components that affect buffering capacity: the first is the pKa (buffering capacity is maximized at a pH close to the pKa of a buffering agent); and the second is molarity (buffering capacity is increased by increasing the concentration of the buffering agent). There is nothing in Watson that teaches or suggests that solutions containing lower concentrations of a buffering agent, and that thus have lower buffering capacities, than those specifically disclosed in Watson would successfully prevent or minimize methacholine hydrolysis. In fact, Watson suggests that higher concentrations of buffering agents are preferred and, thus, teaches away from the lower concentrations set forth in the claims. At best, it might be considered "obvious to try" to see if lower concentrations of buffers might give a stable product. However, "obvious to try" is not a proper standard for determining obviousness under 35 U.S.C. § 103(a).

This is supported by *In re Wiggens*, 158 U.S.P.Q. 199 (C.C.P.A. 1968). The court in *Wiggens* held that claims to a pharmaceutical preparation comprising an old compound at a specified amount were not obvious over a prior art reference teaching the same compound in an amount that was four-fold less than the claimed amount when there was no suggestion in the reference to prepare or administer compositions containing sufficiently greater amounts of the compound. *Id.* at 202.

Applicant respectfully asserts that a worker skilled in the art having regard to Asmus in view of Watson would not have been led to the presently claimed invention. Further, the deficiencies of Asmus and Watson cannot be cured by the disclosure of the Practical Engineering reference, which merely provides a method of sterilization via aseptic filtration. Withdrawal of the rejection is respectfully requested.

Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicant therefore respectfully requests that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicant believes that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

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